Supplementary Discussion

Mechanisms of action of Resiniferatoxin

Resiniferatoxin (RTX) is a compound derived from the plant, Euphorbia resinifera. RTX is a high affinity agonist for transient receptor potential cation channel subfamily V member 1 (TrpV1), an ion channel expressed by nociceptive sensory neurons[63,64]. Our RNAseq data as well as a published database[65] report that neither MeSCs nor differentiated melanocytes express TrpV1. Moreover, we show that buprenorphine, an opioid analgesic, suppresses stress hormone induction, sympathetic nerve activation, and hair greying induced by RTX. Since opioids block pain perception and neurotransmitter release from nociceptive neurons via an independent opioid receptor pathway without affecting RTX binding to the TrpV1 receptor, the hair greying phenotype is not due to non-specific effects the RTX compound might have on other cell types besides nociception induction and subsequent stress responses.

Sympathetic nervous system

The sympathetic nervous system is a part of the autonomic nervous system that regulates many unconscious physiological responses, including heart rate and pupil dilation. The cell bodies of sympathetic neurons are located in the sympathetic ganglia close to the spinal cord, while sympathetic axons extend from the ganglia and innervate all organs including the skin. To evaluate if sympathetic nerve becomes activated, we examined the level of c-FOS (an immediate early transcription factor) in the cell body of sympathetic neurons located at the ganglia.

DREADDs (Designer Receptors Exclusively Activated by Designer Drugs): a chemogenetic tool for neuronal activation

DREADDs are artificial GPCRs that are activated by the synthetic ligand clozapine N-oxide (CNO) and not by any endogenous ligands. In this study, we used a Rosa-lsl-Gq-DREADD line (JAX#026220). Gq protein-coupled receptors play fundamental roles in diverse neuronal

processes, and their activation results in the release of calcium ions from intracellular stores, causing the burst-like firing of neurons[66]. By introducing CNO locally to the skin of TH-CreER; Rosa-lsl-Gq-DREADD mouse, we are able to activate sympathetic nerves innervating specific skin regions directly without any additional stressors and without affecting nociceptive neurons. This experiment allows us to establish that hyper-activation of the sympathetic nerves alone is sufficient to drive MeSC loss and hair greying.

Mechanisms of MeSC depletion under stress

Our results suggest that under stress, MeSCs first undergo aberrant proliferation, followed by differentiation and migration from the niche. The up-regulation of the differentiation program transcriptionally in particular appears to lead these MeSCs to differentiate and permanently depart from their stem cell state. This said, the striking rescue effects of CDK inhibitors (both chemically and genetically) demonstrate that blocking aberrant proliferation is sufficient to prevent MeSCs from undergoing differentiation and migration upon stress, suggesting that differentiation and migration relies on MeSC proliferation to occur as a first step.

Ectopic pigmentation under stress

Ectopic pigmentation can be observed in all histological sections of RTX-injected animals. While this abnormal pigmentation is obvious on tissue sections, and in some cases we do see small pigment aggregates macroscopically on the skin surface, they are not always dark enough to create obvious dark spots. This is likely due to how these ectopic pigments are distributed, how deep they are (i.e., whether they are located in epidermis vs. dermis), and variations in the extent of ectopic pigmentation in each mouse.

References

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